# A STUDY OF CERTAIN METABOLIC INTERMEDIATES IN THE DDT POISONED HOUSEFLY ADULT

BY

PAUL ADOLPH DAHM

A. B., University of Illinois, 1940

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SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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R. UNIV. BIBLIOTHEEK LEIDEN

# A STUDY OF CERTAIN METABOLIC INTERMEDIATES IN THE DDT POISONED HOUSEFLY ADULT

## I. INTRODUCTION

The treatment of the subject of insect physiology by Wigglesworth (1) and in the reviews by Hoskins and Craig (2) and more recently by Craig and Hoskins (3) reveals that very little work has been done on the metabolic intermediates in insects. That which exists originated in comparative studies of the chemistry of muscle contraction. Original discovery of labile phosphorous in vertebrate muscle tissue by Eggletion and Eggleton (4, 5), was followed by the work of Fiske and Subbarow (6, 7), who identified the phosphagen of the Eggletons as a compound containing equimolar amounts of creatine and phosphoric acid. About the same time, however, Meyerhof and Lohmann (8, 9) reported the discovery of another phosphagen in crustacean muscle in which the creatine portion of the molecule was replaced by arginine. In all these studies, insects were omitted from the examples selected. The conspicuous absence of the Class Insecta as examples for these types of studies, as well as the interest in the wing muscles of flying insects, unparalleled in the animal kingdom for their high rate of contraction and relaxation, led Baldwin and Needham (10) to study the phosphorous distribution in the thoracic muscles of adult *Calliphora* and *Lucilia* flies. They obtained a value for phosphoarginine that was considerably lower than that found in many other invertebrates; in addition, the hexose ester content was found to be high compared with frog and rat muscle and the presence of an adenylpyrophosphate was confirmed. Schütze (11) and Heller (12) carried out similar analyses on a number of insects and reported values for total phosphorous and phosphoarginine that exhibited considerable variation.

The recent introduction and phenomenal success of the insecticide DDT (2,2-bis(*p*-chlorophenyl) - 1,1,1 - trichloroethane), has caused much speculation as to its mode of action and biochemical effects. The pattern of DDT poisoning in insects, characterized by violent tremors, suggests certain changes in the body metabolites. Merrill, Savit and Tobias (13) studied the effect of DDT poisoning in the american cockroach (*Periplaneta americana,* Linn.) and found that the poisoned insect did not lose any more weight than did the normal controls, nor did they find any significant dehydration or change in total body non-protein nitrogen content. They did find, however, a marked depletion of total body glycogen and glucose, an increased pyruvate and fat utilization, and a terminal increase in total body acetone and acetoacetic acid content in the DDT poisoned insect.

In this study, the common housefly, *Musca domestica* (Linn.), possessing a known high susceptibility to DDT, was used to determine the effect of DDT on certain metabolic intermediates. In addition, the water content and water loss in normal and DDT poisoned flies were determined.

## II. REARING AND HANDLING OF FLIES

The adult houseflies used throughout this investigation were reared according to the standardized procedure described in the Soap Blue Book (14). The adult flies were fed for five days on a diet of a 10% sugar and water solution before they were used in an experiment. The rapid and reversible anesthetic effect of $CO_2$ (15) was used for anesthetizing the flies during transferring and weighing operations. The use of $CO_2$ was adopted only after it was established that it had no effect upon the products being studied.

Each batch of flies was divided into three approximately equal lots, varying from 100 to 400 flies (2—8 gms.). Lot A consisting of untreated normal flies, was analyzed immediately after weighing and chilling. Lot B, consisting of

untreated normal flies, was transferred, while still immobilized by the $CO_2$ to an uncontaminated one quart cardboard container and placed in the fly rearing room where they remained for 6 hours without food or water. Lot C, consisting of DDT treated flies, was transferred, while still immobilized by the $CO_2$ to a one quart cardboard carton containing a glass plate coated with DDT crystals at the rate of 100 mg. of DDT per square foot and placed in the fly rearing room where they remained for 6 hours without food or water. The flies responded to the DDT within 15-30 minutes and displayed the typical tremors of DDT poisoning for the duration of the 6-hour period. At the end of 6 hours, the flies of Lots B and C were again anesthetized with $CO_2$ and quickly weighed. Losses in weight during the 6 hour period were recorded and the flies chilled in the refrigerator preparatory to analysis.

## III. WATER CONTENT AND WEIGHT LOSSES OF NORMAL AND DDT POISONED FLIES

The average water content of the three lots of flies, after the conditions of treatment described above, was determined by killing the flies with heat and dehydrating the tissue at 110°C. to a constant weight and was found to be: Lot A, 73.5% ; Lot B, 76.8% ; and Lot C, 78.4%.

In the process of weighing lots of flies for analysis and comparing the weight loss of those flies for analysis and comparing the weight loss of those flies which had been untreated and unfed for a period of 6 hours with the weight loss of those flies which had undergone DDT tremors for 6 hours without food or water, it was determined that the untreated and unfed flies lost an average of 14.4% of their original weight in 6 hours and that the DDT treated flies lost an average of 24.6% of their original weight in 6 hours. The difference, or 10.2%, represents a significant additional weight loss due to the effects of DDT poisoning.

## IV. ANALYSIS METHODS

A fractionation process (16) was used for the separation of phosphorylated metabolic intermediates obtained by trichloroacetic acid extraction of aggregates of the whole insects, of lots A, B and C described above, and chemical analyses of certain of these intermediates were carried out using colorimetric methods. The fractionation process separates the components of the acid soluble extract (at pH 8.2) into I, a barium insoluble fraction, II, a barium soluble, alcohol insoluble fraction, and III, a barium soluble, alcohol soluble fraction.

Analyses for total phosphorous, using Fiske and Subbarow's method (17) were made on the original extract, fractions I, II and III, and on the tissue residue.

Analyses for inorganic phosphorous including phosphoarginine, using Fiske and Subbarow's method (17) after the hydrolysis of the phosphoarginine by overnight incubation in acid solution, were made on the original extract and fraction II.

Analyses for "true" inorganic or calcium precipitable phosphorous, using Fiske and Subbarow's methods (7, 17), were made on the original extract and fraction II.

The phosphoarginine content of the original extract and fraction II was obtained by the method of difference (inorganic phosphorous, including phosphoarginine minus "true" inorganic phosphorous) and by the determination of arginine using Sims' (18) modification of the Sakaguchi reaction.

The pyruvic acid content of the original extract (obtained by using an extraction solution of trichloroacetic acid to which had been added iodoacetic acid to a final concentration of 0.2% to prevent an increase of pyruvate) was determined by method "B" of Friedemann and Haugen (19).

Roe's modification (20) of the Seliwanoff reaction was used for the determination of fructose in the original extract

and fraction I.

The micro blood sugar method of Folin and Malmros (21) as modified and adapted to the photoelectric colorimeter by Horvath and Knehr (22) was used for the determination of reducing substances (as glucose equivalents) present in the original extract and fraction II.

A micro-chemical colorimetric determination of total non-protein nitrogen, taken from Umbreit, Burris and Stauffer (16) as modified from the method of Johnson (23), was used for the determination of the non-protein nitrogen in the original extract.

Total phosphorous analyses of the tissue residue remaining after the trichloroacetic acid extraction were made after fusion by the Fiske and Subbarow method (17).

## V. SUMMARY

DDT poisoned housefly adults lose significantly more weight in 6 hours than do normal controls in the same period. This weight loss, which is largely water, reduces the water content of the insect to a critical level and may account for the more rapidly toxic effects of DDT toward the housefly adult than toward other insects such as the american cockroach (*Periplaneta americana* Linn.) and the spotted milkweed bug (*Oncopeltus fasciatus* (Dallas)).

A distribution method has been employed for studying some of the phosphorylated metabolic intermediates occurring in the normal and DDT poisoned housefly adult.

The total phosphorous content of the trichloroacetic acid extract of the normal insect is $140 \pm 11$ mg. % and of the DDT poisoned insect, $155 \pm 11$ mg. %. These values are based on the original wet weight of insect tissue.

The phosphoarginine content of the trichloroacetic acid extract was found to decrease under conditions of DDT poisoning; this decrease of phosphoarginine was accompanied

by a corresponding increase in "true" inorganic phosphorous. The decomposition of phosphoarginine under conditions of DDT poisoning is suggestive of rapidly induced fatigue in the muscle tissue of the poisoned insect.

There is evidence for a partial depletion of glucose and an increased pyruvate utilization in the DDT poisoned housefly adult.

Qualitative evidence was found for the presence of fructose in the trichloroacetic acid extract of the fly tissue.

Although a decrease in the nitrogen content of the trichloroacetic acid extract of the DDT poisoned housefly adult was found, it is felt that additional analyses are needed to establish this point.

Phosphorous analyses of the tissue residue indicate no significant changes under conditions of DDT poisoning.

The overall picture presented by these studies seems to indicate that under conditions of DDT poisoning, the housefly adult catabolizes a variable amount of its food resources but in none of the cases studied does the poisoned insect appear to totally deplete any of the metabolic intermediates studied before expiration. Thus it appears that the variations of the metabolic intermediates between normal and DDT poisoned housefly adults are a result rather than a cause of death from DDT poisoning.

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# VITA

Paul A. Dahm was born November 15, 1917, in Minneapolis, Minnesota. He completed the eighth grade at the Longfellow school in 1931 and graduated from the Oshkosh Public High School in 1935, both at Oshkosh, Wisconsin. In September, 1935, he enrolled at Oshkosh State Teachers College at Oshkosh, Wisconsin, and transferred to the University of Illinois in 1937. He received a Bachelor of Arts degree in Entomology at the University of Illinois in 1940. In September, 1940, he continued his education at the University of Illinois on a first year graduate scholarship in the Department of Entomology, receiving a Master of Arts degree in Entomology at the University of Illinois in June, 1941. During the summers of 1940 and 1941, he was employed as an entomological assistant for the Illinois State Natural History Survey under Professor W. P. Flint. He continued his entomological training on a first year graduate fellowship in Entomology at the University of Illinois in September, 1941. His education was interrupted from 1942 until 1946 by World War II. During these years, he worked as a civilian instructor for the United States Army Air Force Technical Training Command from 1942 to 1943 and then entered military service as a malaria control officer in the United States Navy until February of 1946. In March, 1946, he resumed his graduate studies in Entomology at the University of Illinois. He attended school on a second year graduate fellowship in Entomology from September, 1946, until October, 1947.

He is a member of Phi Beta Kappa, Phi Kappa Phi, the American Chemical Society and an associate member of Sigma Xi. He also belongs to the American Association of Economic Entomologists.

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